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Biomaterial scaffold fabrication techniques for potential tissue engineering applications

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1. Introduction

The dearth of availability of tissues and organs for transplantation as well as inconvenience associated with their transplantation such as donor site morbidity, immune rejection and pathogen transfer led to the emergence of the discipline of tissue engineering. Following its inception in the scientific community since the last two decades, the research and development in this emerging field of tissue engineering and regenerative medicine has progressed in a very rapid rate (Kretlow & Mikos, 2008). Tissue engineering is an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve function of tissue or whole organ (Chen et al., 1997; Bhatia et al., 1999; Huang & Ingber, 2000; Chiu et al., 2000; 2003). Tissue engineering has emerged as a new method involving the combining of cells, scaffold, and bioactive agents to fabricate functional new tissue to replace damaged tissue (Flanagan et al., 2006; Chen et al., 2002; Solchaga et al., 2001).

A well-designed three-dimensional scaffold is one of the fundamental tools to guide tissue formation *in vitro* and *in vivo*. Frontiers areas in medicine is changing rapidly from utilizing synthetic implants and tissue grafts to a tissue engineering approach that uses degradable porous material scaffolds integrated with biological cells or molecules to regenerate tissues (Hollister et al., 2005). Therefore, the selection of scaffold is important to enable the cells to behave in the desired manner to generate tissues and organs of the desired shape and size.

Characteristics of scaffolds

- (1) Biocompatibility, to avoid unwanted host tissue responses to the implant (Ma, & Langer, 1999).
- (2) It should have the excellent surface chemistry to allow attachment, migration, proliferation, and differentiation of the cells (Mandal and Kundu, 2008b; 2009a; 2009b).
- (3) Interconnected pores with proper pore size to support cell infiltration and vascularization (Hutmacher 2001; Karageorgiou & Kaplan, 2005).
- (4) Controlled biodegradability to aid the formation of new tissue (Cima et al., 1991; Kweona et al., 2003).

(5) Adequate mechanical properties to maintain the structure and function immediately after implantation and during remodelling of the implants (Karande & Agrawal, 2008; Kim et al., 2000; Lee et al., 2001).

(6) It should acquire the sufficient mechanical properties to provide the better environment for cells, deliver inductive molecules or cells to the repair site and provide cues to control the structure and function of newly formed tissue (Hutmacher 2000; 2001).

(7) It should support the formation of ECM by promoting cellular functions, and have the ability to provide the bimolecular signals to the cells (Ito et al., 2003).

(8) Scaffolds used for tissue engineering should mimic in part the structure and biological function of the extracellular matrix (Ma, 2008; Li & Shi, 2007).

Many techniques have been developed to fabricate three-dimensional porous architectures to fill this role, such as by particle leaching (Ma & Langer, 1999; Lu et al., 2000), phase separation (Sachlos & Czernuszka, 2003; Smith et al., 2006) and textile technology. However, scaffolds fabricate by these techniques do not adequately mimic the structure of the natural extracellular matrix in terms of architecture, which may be one of the reasons for suboptimal outcome in generating functional tissues (Lavik, & Langer, 2004; Laurencin et al., 1999; Hutmacher 2001). Therefore, new designs and manufacture technologies are required to improve the function and architecture of scaffolds. This chapter discusses the different scaffold fabrication methodologies/techniques utilizing several synthetic and natural polymers, including solvent casting, particulate leaching, gas foaming, phase separation, electrospinning, porogen leaching, fiber mesh, fiber bonding, self assembly, rapid prototyping, melt molding, membrane lamination and freeze drying.

2. Fabrication techniques

In the body, cells and tissue are organized into three-dimensional architecture. To engineer these functional tissue and organs, scaffolds have to be fabricated by different methodology to facilitate the cell distribution and guide their growth into three-dimensional space. The main techniques for scaffolds fabrication are mentioned here.

2.1 Solvent casting

Solvent casting property for the scaffolds preparation is very simple, easy and inexpensive. It does not require any large equipment; it is totally based upon the evaporation of some solvent in order to form scaffolds by one of the two routes. One method is to dip the mold into polymeric solution and allow sufficient time to draw off the solution; as a result a layer of polymeric membrane is created. Other method is to add the polymeric solution into a mold and provide the sufficient time to evaporate the solvent that create a layer of polymeric membrane, which adhere to the mold (Mikos et al., 2004).

One of the main drawbacks of this technique, the toxic solvent denatures the protein and may affect other solvents. There is a possibility that the scaffolds designed by these techniques may also retain some of the toxicity. To overcome these problems scaffolds are fully dried by vacuum process to remove toxic solvent. However, this is very time consuming technique and to overcome these problems some researchers have combined it with particulate leaching techniques (Mikos et al., 1993a; 1993b; 1996) for the fabrication of scaffolds.

2.2 Particulate-leaching techniques

Particulate leaching is one of the popular techniques that are widely used to fabricate scaffolds for tissue engineering applications (Ma & Langer, 1999; Lu et al, 2000). Salt, wax or sugars known as porogens are used to create the pores or channels. Here salt is grounded into small particles and those particles that have desired size are poured into a mold and filled with the porogen. A polymer solution is then cast into the salt-filled mold. After the evaporation of the solvent, the salt crystals are leached away using water to form the pores of the scaffold.

The process is easy to carry out. The pore size can be controlled by controlling the amount of porogen added, the size and shape of the porogen (Plikk et al., 2009). The particulate leached scaffold possesses pore size (~500 μm), percentage porosity (around 94-95%) and desired crystallinity. The advantage of this method is the requirement of very less amount of polymer to fabricate the scaffold. However, certain critical variables such as pore shape and inter-pore openings are not controlled. To conquer these drawback new technologies, are being developed.

2.3 Gas foaming

Many of the fabrication techniques require use of organic solvents and high temperature. The residues that remains after completion of process can damage cells and nearby tissues. This may also denature the biologically active molecules incorporated within the scaffolds. The gas foaming scaffold fabrication techniques does not require the utilization of organic solvents and high temperature.

This technique uses high pressure carbon dioxide gas for the fabrication of highly porous scaffolds. The porosity and porous structure of the scaffolds depend upon the amount of gas dissolved in the polymer. This process involves exposing highly porous polymer with carbon dioxide at high pressure (800 psi) to saturate the polymer with gas (Sachlos & Czernuszka, 2003). Under this condition, dissolved carbon dioxide becomes unstable and will phase separates from the polymer. The carbon dioxide molecule becomes cluster to minimize the free energy; as a result pore nucleation is created. These pores cause the significant expansion of polymeric volume and decrease in polymeric density. A three-dimensional porous structure (scaffolds) is formed after completion of foaming process.

The porosity of the scaffolds is controlled by the use of porogens like sugar, salts and wax (Ikada, 2006). The polymer (eg, PLGA) that expands in foaming process fused together around the porogen to create a continuous polymeric matrix, and also entrap any other molecule which is present in the mixture. The mix polymer and porogen are exposed to high pressure until they have completed its saturation with carbon dioxide, followed by foaming process porogen is removed and a highly interconnected pore structure is formed (Huang & Mooney, 2005).

2.4 Phase separation

Phase separation technique for scaffolds designing requires temperature change that separates the polymeric solution in two phases, one having low polymer concentration (polymer lean phase) and other having the high polymer concentration (polymer rich phase). Polymer is dissolved in phenol or naphthalene, followed by dispersion of biologically active molecule in these solutions. By lowering the temperature liquid-liquid

phase is separated and quenched to form a two phase solid and the solvent has removed by extraction, evaporation and sublimation (Mikos et al., 2004) to give porous scaffolds with bioactive molecules integrated in to that structure (Sachlos & Czernuszka, 2003; Hua et al., 2002).

An appropriate liquid-liquid phase separation is critical for the preparation of nanofibers and does not occur in all solvents that's why selection of solvent and phase separation temperature is crucial for the formation of nanofibers. When the condition are favourable, liquid-liquid phase separation produce three dimensional fibrous structure with nano scaled architecture similar to that of collagen type I, and used in various biomedical applications.

Advantage of the phase separation technique is that, it can easily combine with other fabrication technology (Particulate leaching) to design three dimensional structures with control pore morphology. It can also be combined with rapid prototyping to create nano fibrous scaffolds for tissue engineering applications (Smith et al., 2006).

2.5 Electrospinning

The electrospinning technique for the scaffolds designing utilizes the electrostatic force for the production of polymeric fiber ranging from nanoscale to microscale. This process is control by high intensity electric field between two electrodes having electric charges of opposite polarity. One electrode is placed in the polymer solution and other is placed in collector. Generally polymer solution is pumped as result in forming a drop of solution. Afterwards, electric field is generated, which intends to produce a force, due to this the droplets results to overcome the surface tension of the solution. A jet of polymer is ejected, which produces the fibers, same instant the solvent starts evaporating due to jet formation and continues after the nanofibers are deposited to collector.

More than 200 polymers are used for electrospinning like silk fibroin (Zarkoob et al., 2004; Sukigara et al., 2003; Jin et al., 2004), collagen (Mathews et al., 2002), chitosan (Ohkawa et al., 2004), gelatin (Ma et al., 2005) etc. In the field of tissue engineering electrospinning technique is applied for the preparation of nanofiber scaffold design. The process is very versatile in terms of use of polymers, non-invasive and does not require the use of coagulation chemistry or high temperature for fiber generation. Basically, in this process a high voltage is used to create an electrically charged jet of polymer solution or melt, which forms polymer fiber after drying or solidification (Reneker and Chun, 1996; Doshi and Reneker, 1995).

One of the main advantages of this technique is that it can produce the scaffold with main structural feature suitable for growth of the cell and subsequent tissue organization (Li & Tuan, 2009; Liang et al., 2007; Leong et al., 2008). It can produce the ultra fine fibers with special orientation, high aspect ratio, high surface area, and having control over pore geometry. These characteristics are favorable for better cellular growth for *in vitro* and *in vivo* because they directly influence the cell adhesion, cell expression, and transportation of oxygen, nutrients to the cells. This provides spatial environment for the growth of new tissue with appropriate physiological functions. Cell seeding is the main problem of electrospining technology. This is overcome by sacrificial biopolymer or cryospinning, which allows creating the hole of desired size in electrospun matrices (Baker et al., 2008; Leong et al., 2008).

The nano fibrous scaffolds are widely used in biomedical application for scaffolds preparation for tissue engineering (Ma et al., 2005; Yang et al., 2005), wound dressing (Kim et al., 2000), artificial blood vessels (Ma et al., 2005), protective clothing material (Lu and Ding, 2008), drug release membrane (Katti et al., 2004; Chew et al., 2005; Venugopal et al., 2008), nanotube material, chemical catalytic apparatus, bio-transplant material, and hydrogen storage tank for fuel cell (Cho et al., 2003).

The nanofibrous scaffolds are prepared for the biomedical application such as hydrophilicity, mechanical strength, biodegradability, biocompatibility, interaction of cells, which are controlled by chemical composition of the material (Madurantakam et al., 2009). On the basis of that by selecting and adjusting the combination of proper component ratio, property of electrospinning scaffolds can be customized with desired function.

2.6 Porogen leaching

Porogen leaching is one of the most common methods used for preparation of scaffolds with controlled porosity. The particulate leaching method is totally based upon the dispersion of porogen (salt, sugar and wax) either in liquid particulates or powdered materials (Hou et al., 2003; Lee et al., 2004; Nazarov et al., 2004; Vepari & Kaplan, 2007) by the process of evaporation, cross linking or other reaction liquid may be solidified. These porogens act as place holder for pore and interconnection of the pores in the actual scaffolds fabrication technique. Highly porous scaffold with porosity up to 93 % and pore diameter up to 500 micrometers can be prepared by using this technique (Mikos et al., 1993 a; b).

Main objective of this technique is the realization of bigger pore size and increase pore interconnectivity. Main advantage of this technique is its simplicity, versatility and easy to control the pore size and geometry. Pore geometry is control by the selection of the shape for specific porogen agent, where as pore size is control by sieving the porogen particle to the specific dimensional range (Mano et al., 2007). One of the main drawbacks of this technique is that it can only produce thin wafers or membrane up to 3 mm thick and very difficult to design the scaffolds with accurate pore inter-connectivity (Moore et al., 2004).

2.7 Fiber mesh

Fiber mesh technique for scaffold fabrication consists of individual fiber either woven or interweave into three dimensional pattern of variable pore size (Martins et al., 2009). PGA is the first biocompatible and biodegradable polymer to spun into the fiber and used as a synthetic suture thread. It is prepared by the deposition of polymer solution over a nonwoven mesh of another polymer followed by subsequent evaporation (Ikada, 2006).

Main advantage of this technique is to provide the large surface area for cell attachment and rapid diffusion of nutrient that is favourable for cell survival and growth (Chen et al., 2002). However one of the main drawbacks of this technique is lack of structural stability which can partly be overcome by hot drying of PLLA fiber to improve the structure orientation and crystallinity.

2.8 Fiber bonding

Fiber bonding technique for scaffold fabrication is developed by Mikos and his coworkers (Mikos et al., 1993a). Synthetic polymer (PLLA) was dissolved in chloroform followed by non-woven mesh of PGA fiber had added. Subsequently solvent was removed by

evaporation as a result a composite material, which consists of non-bonded PGA fiber, embedded in PLLA matrix is formed (Chen et al., 2002). The scaffolds were fabricated by bonding a collagen matrix to PGA polymers with threaded collagen fiber stitches (Eberli et al., 2009). Fiber bonding occurs during post treatments at a temperature above the melting temperature of PGA. As a result PLLA matrix of the composite has removed by dissolving in methylene chloride agent (Sachlos and Czernuszka, 2003) utilizing the fact that PGA is insoluble in this solvent.

This process yields the scaffolds of PGA fiber that is bonded together by heat treatment. PGA mesh provides the high porosity and surface area to polymer mass ratio (Mooney et al., 1996). This provides the mechanical stability and allows the tissue ingrowths. One of the main advantages of using the fiber is its large surface area, which is suitable for scaffolds applications. Therefore, it provides the more surface area for cells attachment and sufficient space for the regeneration of extracellular matrix (Moroni et al., 2008)

2.9 Self assembly

Self assembly is the spontaneous organization of the molecule into well defines into an ordered structure required for specific function (Zhang, 2003). Self assembly of natural or synthetic molecule produced nanoscale fibers known as nanofibers.

Amphiphilic peptide sequence is a common method for the fabrication of 3D nanofibrous structure for tissue engineering. In aqueous solution the hydrophobic and hydrophilic domains within these peptides interact together with the help of weak non covalent bonds (Joshi et al., 2009; Zhang et al., 2006) (eg. Hydrogen bond, Van der Waals interactions, ionic bond and hydrophobic interaction) this produces distinct fast recovering hydrogel, with the hydrophobic interactions as the molecules come together.

Instead of peptides synthetic polymer nanofibers are also prepared by self assembly of diblock polymers (AxB) when the two blocks separate from one another in bulk due to their incompatibility, the volume formation of A and B can be controlled to obtain B domain of cylindrical shape with nanoscale diameter that is embedded into matrix A. Polymeric dendrimers can also self-assemble into nanofibers (Liu et al., 1996; 1999).

The di- and tri-block peptide ampholites (PAs) are designed that are self-assembled into a rod-like architecture. So a new technique for the self-assembly of PAs into nanofibers by controlling pH and by engineering the peptide head group of the PAs is developed (Hartgerink et al., 2001).

According to Hartgerink et al., (2002) and Tambralli et al., (2009) the salient features for synthesis of the PA involve the following:

1. Phosphoserine residue incorporation to increase hydroxyapatite mineralization.
2. RGD (Arg-Gly-Asp) peptide incorporation to enhance integrin-mediated cell adhesion.
3. Four consecutive cystine residues incorporation forming inter-molecular disulfide bonds that polymerizes to provide improved structural stability.
4. Flexible linker region incorporation consisting of three glycine residues to provide flexibility to the head group.

By virtue of the modifications in the structure of the PA enables a variety of self-assemblies including layered and lamellar structures and by its reversibility properties provides flexibility to the system. Therefore, the self-assembly technique shows designing potential novel scaffolds for tissue engineering applications.

Self assembly shows several advantages over the electrospinning because it produces the much thinner nanofiber with very thin diameter (Ma, 2008). The fabricated nanofibers have amino acid residues that may be chemically modified by the addition of bioactive moieties. Other advantage of this technique is to avoid the use of organic solvent and reduce the cytotoxicity because it is carried out in aqueous salt solution or physiological media (Ma et al., 2005). Main disadvantage of this technique is its complicated and elaborated process.

2.10 Rapid prototyping (RP)

RP is also called as solid free-form technique. This technique is more advanced technique for scaffold fabrication. It is computer controlled fabrication technique. It can rapidly produce 3D object by using layer manufacturing method. RP technique generally comprises the design of scaffold model by using the computer added design (CAD) software, which is then expressed as a series of cross section (Lin et al., 2008, Woodfield et al., 2009). Corresponding to each cross section RP machine lays down a layer of material starting from the bottom and moving up a layer at a time to create the scaffolds. In typical example, image of bone defect in a patient can be taken and develop 3D CAD computer model. The computer then can reduced the model to slice or layers. The 3D objects are constructed layer by layer by using RP techniques such as fused deposition modeling (FDM), selective laser sintering (SLS), 3D printing (3D-P) or stereolithography.

Now a day RP is an efficient way for generating the scaffolds of desired property, other advantage of this technique is to produce the parts with highly reproducible architecture and compositional variations. RP has advantage over other fabrication techniques, it has ability to control matrix architecture (size, shape, inter connectivity, branching, geometry and orientation) yielding biomimetic structure, that varying in design and material composition. It has ability to control the mechanical property, biological effects and degradation kinetics of scaffolds (Kai et al., 2009; Hutmacher et al., 2000; 2001).

RP technique can easily be integrated with the imaging technique to produce the scaffolds that are customized in size and shape allowing the tissue engineered graft to be modified for particular applications or for individual patient. One of the main drawbacks of this technique is achieved low resolution by current systems and types of polymeric materials that are used for this technique

2.11 Melt molding

A large number of techniques are discussed for the fabrication of scaffolds. These scaffolds fabrication techniques are developed to control the pore interconnectivity and geometry, which are important for the exchange of nutrient/waste from pore to pore. Melt molding process involves the filling of teflon mould with PLGA powder and gelatin microspheres of specific diameter followed by heating the mould above the glass transition temperature of PLGA while applying pressure to the mixture (Thompson et al., 1995a, 1995 b). This action causes the PLGA particle to attach together. Once the mould is removed gelatin microspheres is dissolved by immersing the mixture into water and scaffolds are then dried. Scaffolds produced by this technique assume the shape of the mould. Melt molding process was modified to incorporate short fiber of hydroxyapatite (HA). Uniform distribution of HA fiber throughout the PLGA scaffolds could only be achieved by using the solvent casting

technique to prepare the composite material of HA fiber, PLGA matrix and gelatin or salt porogen, which are used in melt molding process (Hou et al., 2003).

2.12 Membrane lamination

Membrane lamination is another SFF-like technique used for constructing three-dimensional biodegradable polymeric foam scaffolds with precise anatomical shapes. Membrane lamination is prepared by solvent casting and particle leaching and introducing peptide and proteins layer by layer during the fabrication process. The membranes with appropriate shape are soaked with solvent, and then stacked up in three-dimensional assemblies with continuous pore structure and morphology (Maquet & Jerome, 1997). The bulk properties of the final 3D scaffolds are identical to those of the individual membrane.

This method generates the porous 3D polymer foams with defined anatomical shape, since it is possible to use the computer assisted modeling to design the template with desired implant shape. The disadvantage of this technique is that layering of porous sheets, result in lesser pore interconnectivity (Hutmacher et al., 2000; 2001) and other disadvantage of this technique is that it is a time consuming process since only thin membrane can be used in this process.

2.13 Freeze drying

Freeze drying technique is used for the fabrication of porous scaffolds (Whang et al., 1995; Schoof et al., 2001). This technique is based upon the principle of sublimation. Polymer is first dissolved in a solvent to form a solution of desired concentration. The solution is frozen and solvent is removed by lyophilization under the high vacuum that fabricate the scaffold with high porosity and inter connectivity (Mandal & Kundu, 2009 a, b). This technique is applied to a number of different polymers including silk proteins (Vepari & Kaplan, 2007, Altman et al., 2003) PGA, PLLA, PLGA, PLGA/PPF blends. The pore size can be controlled by the freezing rate and pH; a fast freezing rate produces smaller pores. Controlled solidification in a single direction has been used to create a homogenous 3D-pore structure (Schoof et al., 2001).

Main advantage of this technique is that, it neither requires high temperature nor separate leaching step. The drawback of this technique is smaller pore size and long processing time (Boland et al., 2004). A schematic diagram for scaffold fabrication from silk protein by freeze drying technique is given as an example in figure 1 (Mandal & Kundu, 2008a; 2008b; 2009b; Kundu et al., 2008).

Methods	Merits	Demerits	References
Solvent casting/ particulate leaching	Control over Porosity, pore size and crystallinity	Limited mechanical property, residual solvents and porogen material	Ma, 2007; Xiang et al., 2006
Porogen leaching	Controlled over porosity and pore geometry	Inadequate pore size and pore interconnectivity	Mano et al., 2007
Gas foaming	Free of harsh organic solvents, control over porosity and pore size	Limited mechanical property, inadequate pore interconnectivity	Ikada., 2006
Self assembly	Control over porosity, pore size and fiber diameter	Expensive material, complex design parameters	Zhang et al., 2003; 2006
Electrospinning	Control over porosity, pore size and fiber diameter	Limited mechanical property, pore size decrease with fiber thickness	Liang et al., 2007
Phase separation	No decrease in the activity of the molecule	Difficult to control precisely scaffold morphology	Smith et al., 2006
Rapid prototyping	Excellent control over geometry, porosity, no supporting material required	Limited polymer type, highly expensive equipment	Hutmacher et al., 2000; 2001
Fiber mesh	Large surface area for cell attachment, rapid nutrient diffusion	Lack the structural stability	Chen et al., 2002
Fiber bonding	High surface to volume ratio, high porosity	Poor mechanical property, limited applications to other polymers	Mooney et al., 1996
Melt molding	Independent control over porosity and pore size	Required high temperature for non amorphous polymer	Thompson et al., 1995 a; b
Membrane lamination	Provide 3D matrix	Lack required mechanical strength, inadequate pore interconnectivity	Maquet & Jerome, 1997
Freeze drying	High temperature and separate leaching step not required	Small pore size and long processing time	Boland et al., 2004; Mandal & Kundu, 2008

Table 1. Merits and demerits of different fabrication techniques

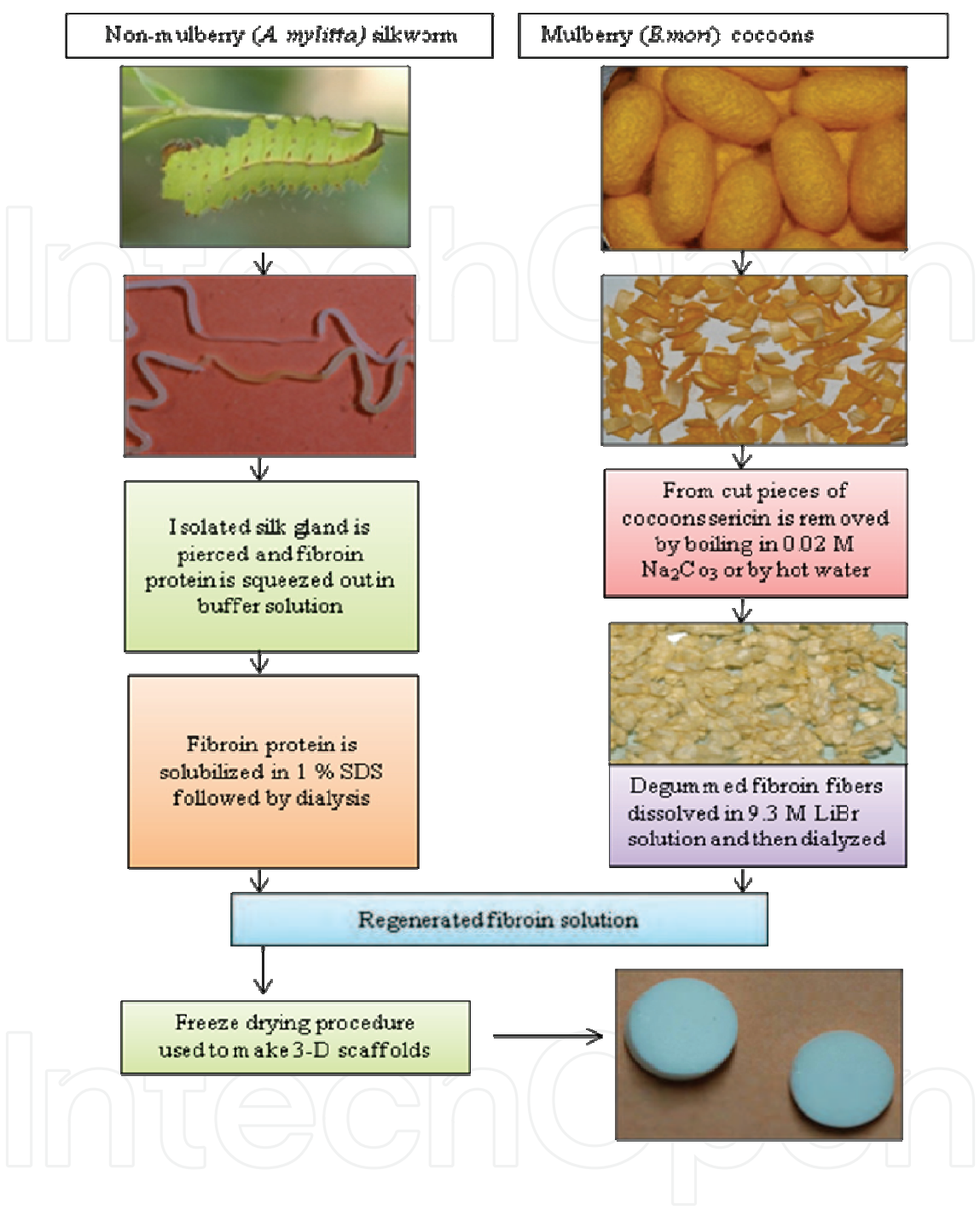


Fig. 1. Schematic diagram of scaffold fabrication from silk fibroin protein by freeze drying technique. The silk fibroin proteins may be obtained from two different silkworm sources either from the silk gland of non mulberry late 5th instar silkworm larva as an example from Indian tropical tasar silkworm (*Antheraea mylitta*). It is difficult to obtain sufficient amount of fibroin from cocoon sources of non mulberry (Mandal & Kundu, 2008a; 2008b; 2009 a) or from cocoons of mulberry silkworm, *Bombyx mori* (Altman et al., 2003; Mandal and Kundu, 2009b; Kundu et al., 2008).

3. Conclusion

Scaffolds are used to provide sites for cells attachment, proliferation, differentiation and migration by up regulating and down regulating the synthesis of protein and growth factors. They provide mechanical support, deliver inductive molecules or cells to the repair site. Scaffolds also provide cues to control the structure and function of newly formed tissue. Recently decellularized extracellular matrix has been suggested as a scaffold for heart valve tissue engineering or direct implantation. However, cell removal damages the physical and biochemical properties of the valve leaflet structure. Matrix /polymer hybrid scaffold with improved biomechanical characteristics may be advantageous for many tissue engineering aspects such as heart valve tissue engineering (Hao et al., 2008). Equilibrium is needed between highly porous scaffolds that allow rapid tissue ingrowths and minimize diffusion limitations and less porous materials that retain both construct shape and the ability to bear mechanical loads in a complex biochemical and mechanical environment.

The incorporation of the bioactive molecules like the growth factor, enzymes, ECM proteins, DNA is another vital consideration while designing the scaffolds. The fabrication methodologies that do not inactivate the biological activity of the bioactive molecule within the scaffolds need to be used judiciously. Scaffolds can be made by different types of fabrication techniques. The fabrication techniques provide the regular architectures; allow mechanical and finite element analyses to be carried out, which permit quantification of suitable mechanical and chemical microenvironments for cells and tissues assisting in the development of the next generation of tissue engineered scaffolds. The fabrication technique for the tissue engineered scaffold is directly related to the bulk and surface properties of the polymer and the proposed function of the scaffold. While each technique has its inherent merits and demerits, the appropriate selection of the methodologies must satisfy the requirements of the specific tissue to be repaired or engineered. Another hindrance in the tissue engineering research and scaffold design is the ignorance of the researchers on the possible interactions that exists between different cell types, such as the communication between the scaffold and the cells seeded within, cells and the different soluble factors, the typical behaviour of cells under the physical forces (shear, compression and vibration forces)

The different techniques are developed to improve the current scaffold design by controlling the regular pore size, pore interconnectivity, porosity and pore characterizations that is suitable for rapid nutrient diffusion and cell attachment. Table 1 shows the most commonly used scaffolds fabrication technologies for potential tissue engineering applications indicating their merits and demerits of each technique. Some references given below in this chapter allow interested readers to go through the detail of fabrication techniques. Recent advances in both computational topology design (CTD) and solid free-form fabrication (SFF) have made it possible to create scaffolds with controlled architecture (Hollister, 2005). Finally, future directions are utilization of modelled scaffolds with *in vivo* experimentation to optimize tissue-engineering treatments, and coupling scaffolds with cells, genes and proteins optimized to create smart scaffolds for tissue regeneration and controlling their delivery through the scaffold with spatial and temporal resolution.

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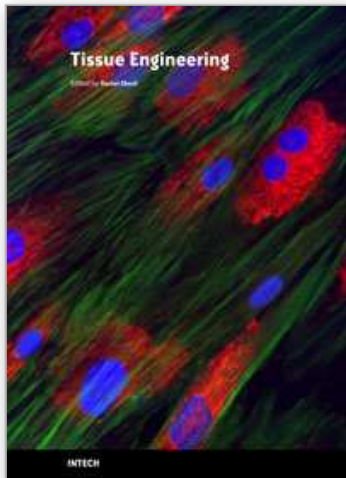
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The Tissue Engineering approach has major advantages over traditional organ transplantation and circumvents the problem of organ shortage. Tissues that closely match the patient's needs can be reconstructed from readily available biopsies and subsequently be implanted with minimal or no immunogenicity. This eventually conquers several limitations encountered in tissue transplantation approaches. This book serves as a good starting point for anyone interested in the application of Tissue Engineering. It offers a colorful mix of topics, which explain the obstacles and possible solutions for TE applications.

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